Chapter

Investigation and Management of Endocrinopathies in Thalassaemia Major

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Abstract

A combination of sub-therapeutic chelation and subsequent iron overload are regarded as the principal drivers of endocrine dysfunction in thalassaemia. The clinical presentation of endocrine complications and their timing of onset can be highly variable, in part due to population heterogeneity but also variation in chelation strategies. Endocrinopathies commonly associated with thalassaemia include: growth delay; pubertal delay; gonadal dysfunction; thyroid disorders; parathyroid and adrenal gland impairment; impaired bone metabolism; and type 2 diabetes mellitus. In this chapter we summarise the main presentations of endocrine disorder in thalassaemia, summarising their epidemiology, clinical presentation and pathophysiologic basis. Furthermore, we review screening, monitoring and treatment strategies, with particular regard to the UK Thalassaemia Society's 2016 National Standards.

Keywords: thalassaemia major, beta thalassaemia, endocrinopathy, endocrine dysfunction, iron overload, puberty, thyroid, adrenal, diabetes mellitus, bone density

1. Introduction

The inherited haemoglobinopathy thalassaemia major (also known as beta thalassaemia major) results from homozygous carriage of mutations at the beta-globin locus, resulting in defective haemoglobin synthesis and a severe hypochromic, microcytic anaemia. Epidemiologically, the largest incidence of thalassaemia major is in the Mediterranean countries and the Middle East, but demographic change and international migration have resulted in it posing a global health issue. Life expectancy has been radically increased by the advent of combined transfusion and chelation therapy, but this regimen is complicated by both citrate toxicity and the development of endocrine complications secondary to destructive haemosiderin deposition in glandular tissues, particularly during adolescence and young adulthood [1]. Pathologic iron deposition is concentrated in cardiac tissue, the liver parenchyma and endocrine glands [2], with the development of multiple endocrinopathies first being reported by Bannerman and colleagues in 1967 [3]. Determining the prevalence and onset patterns of endocrine disease in thalassaemia major remains a challenge, despite their high frequency; primarily, this related to variation in exposure to chelation therapy, compliance with chelation, and improved survival shedding light on new disease phenomena [4–8].

Chronic anaemia—and subsequent tissue hypoxia—results in compensatory increases in erythropoiesis and gastrointestinal iron absorption. In conjunction with regular blood transfusion, these processes conspire to produce massive iron deposition in thalassaemia. Iron is regulated exclusively at the level of absorption, with no excretory mechanism. Chelation therapies are available, but challenges in their administration, including via the parenteral route, and need for regular

	Routine: annual	**Routine: other interval**	**Specific circumstances**
Glycaemic Control	Non-DM patients*: OGTT and fructosamine DM: fructosamine and review as per NICE 9 Key Care Processes**	DM: home capillary blood glucose as per individual management plan	• IFG or IGT: fructosamine at 6-monthly intervals • Symptomatic hyperglycaemia: random plasma glucose ± ketones to exclude DKA
Thyroid Function	Thyroid Function Tests (TFTs)†: free TSH, free T3, free T4		• Clinical evidence of thyroid dysfunction: random TFTs
Puberty	Systematic clinical assessment from age 10: Tanner staging		
Gonadal Function	Annual clinical assessment‡ Men: annual morning testosterone Women: if normal menstrual history, no further investigations indicated	Male: if low testosterone ➔ measure LH/FSH/SHBG	Female: if oligomenorrhoea/amenorrhoea develop ➔ measure LH/FSH/oestradiol. Specialist endocrine review advised before initiation of hormone replacement.
Growth		6-monthly: height/weight/height velocity from diagnosis to attainment of final adult height	If concerns regarding growth delay: bone age estimation (wrist plain radiographs) at 1–2 year intervals Faltering height velocity: growth hormone stimulation test
Bone Metabolism	Vitamin D level•	6-monthly••: calcium/phosphate/ALP ➔ PTH if calcium level low	
Adrenal Function	Annual morning cortisol level		

Key: OGTT: oral glucose tolerance test DM: diabetes mellitus IFG: impaired fasting glycaemia IGT: impaired glucose tolerance DKA: diabetic ketoacidosis PTH: parathyroid hormone.
ALP: alkaline phosphatase LH: luteinizing hormone FSH: follicle-stimulating hormone SHBG: sex-hormone binding globulin.
**From puberty, or age 10 years if family history of DM.*
†From age 12 years.
••From age 12 years.
***NICE 9 Key Care Processes: glycaemic control (via Fructosamine); blood pressure; cholesterol level; retinal screening; foot checks; urinary albumin testing; serum creatinine testing; weight monitoring; smoking status check.*
‡Including, for female patients, menstrual history; for male patients: history of impotence.
•From age 2 years. UKTS (2016) guidelines recommend ~80 nmol/L optimal target following supplementation.

Table 1.
Summary of endocrine investigations in thalassaemia major [after UK Thalassaemia Standards, 2016].

blood monitoring, hamper their acceptability. The primary endocrine complications found in thalassaemia, in the order reviewed in this chapter, are: disorders of growth; sexual development and fertility; abnormal bone mineralisation; diabetes mellitus; hypothyroidism; and hypoadrenalism [9].

We have previously published an online Review of Endocrine Disorders in Thalassaemia in 2014 (Open Journal of Endocrine and Metabolic Diseases, 2014, 4, 25–34). In this book chapter, we have updated all the latest evidence and discuss current thoughts & details of the multi-system endocrine involvement in Thalassaemia. Finally, **Tables 1** and **2** summarise the investigations and treatment of such patients.

Glycaemic Control	• Impaired glucose regulation (IFG/ IGT) or Non-insulin treated Diabetes: Intensify chelation therapy, consider using combination chelation regimens • Diabetes: Referral to diabetes specialist. Managed according to NICE treatment targets and recommendations for type 1 and type 2 diabetes	In patients with symptoms/signs of Diabetic Ketoacidosis (DKA) who are acutely unwell and plasma glucose>12 mmol; measure blood or urinary ketones
Thyroid Function	• Hypothyroidism: thyroxine replacement	✦Hypothyroidism may impair cardiac function/rhythm
Puberty	• Suspicion of pubertal delay: Referral to paediatric endocrinologist	
Gonadal Function	• Hypogonadism: hormone (oestrogen/ testosterone) replacement • Females: combined oral contraceptive pill (when contraception is also required, or "post-menopausal" replacement regime)	✦Specialist endocrine review advised before initiation of hormone replacement.
Growth	• Positive growth hormone stimulation test: may consider GH therapy in childhood✣	
Bone Metabolism	• Vitamin D replacement if required • Bisphosphonates: considered for patients with low BMD for age‣, fragility fractures and/or falling BMD despite adequate vitamin D levels • Denosumab▷: advisable that treatment is supervised by a clinical with experience and interest in osteoporosis	• Bisphosphonate: should be reviewed after a maximum of 5 years for oral agents and 3 years for IV agents. Bisphosphonate 'holiday': is recommended after the above intervals for a period of 2–3 years
Adrenal Function	• Adrenal insufficiency: Hydrocortisone supplementation	✦Adrenal dysfunction may be subacute (during acute illness), consider adrenal support, even before formal proof of insufficiency is available

Key: DM: diabetes mellitus IFG: impaired fasting glycaemia IGT: impaired glucose tolerance DKA: diabetic ketoacidosis BMD: Bone Mineral Density GH: growth hormone.
✦*Note.*
‣*Z-score < −2.0 if premenopausal or under 50, t-score < −2.5 if post-menopausal or over 50.*
▷*Anti-RANKL monoclonal antibody.*
✣*some evidence for GH therapy in childhood only.*

Table 2.
Summary of endocrine treatments in thalassaemia major [after UK Thalassaemia Standards, 2016].

2. Growth and development in thalassaemia

From the foetal, through to infantile, pre-pubertal period and puberty, children with thalassaemia exhibit delayed growth [9]. It is estimated that 20–30% of these children and adolescents are affected by growth hormone (GH) deficiency [10]. In the remainder of thalassaemic patients without overt growth hormone deficiency, provocative testing—for example clonidine or glucagon stimulation tests—suggests that peak GH levels are lower than seen in constitutive short stature. Dhouib et al. recently showed a 35% incidence of GH deficiency in a Tunisian paediatric cohort [11]. Multiple causes for growth failure have been posited. These include features directly related to iron overload, including free radical toxicity [12]; damage to other endocrine axes, including the GH/Insulin-like growth factor (IGF-1) axis [13], pubertal delay and hypothyroidism; and complications of therapy, including chelation agent, particularly desferrioxamine, toxicity [14]. Hepatic cirrhosis, anaemia and zinc deficiency have also been implicated [15].

The anterior pituitary gland is particularly vulnerable to oxidative stress caused by free radicals, with even modest levels of iron deposition, detected by magnetic resonance imaging (MRI), disrupting its function [12]. Comparative studies of diurnal hormone secretion suggest that the 24-hour profile of GH secretion, and the response growth hormone releasing hormone (GHRH, secreted by the arcuate nucleus of the hypothalamus), in thalassaemic patients is similar that of children with idiopathic short stature [16]. It is posited that thalassaemia major may be associated with increased somatostatin tone, with subsequent disruption of GH secretion [17].

Thalassaemia major may be characterised by relative growth hormone deficiency, implied by the low levels of serum IGF-1 but normal GH reserve seen in patients. The positive therapeutic response seen with exogenous GH supplementation implies that, at the post-receptor level, this resistance may only be partial [18]. Anaemia, ineffecient erythropoiesis and chelation therapy also inhibits linear growth in children with thalassaemia major. Desferrioxamine and pathologic iron deposition are proposed to disrupt local IGF-1 production and paracrine signalling at the growth plate [14], resulting in inhibition of cellular proliferation and mineral deposition. Truncal shortening and abnormal body proportions are frequently observed, and have been attributed to the disease process itself, compounded by iron and desferrioxamine toxicity [18]. Limited evidence is emerging that these phenomena may be at least partly contingent on the timing of initiation of chelation and its route of administration. Soliman and colleagues reported a cross-sectional cohort analysis of beta thalassaemia patients commenced on oral iron chelation (OIC) with desferoxamine either before (n = 15) or after (n = 40) attaining final adult height. In this small study, pre-pubertal initiation of OIC was correlated with increased final adult height, in parallel with a lower overall incidence of endocrinopathies and reduced hepatic iron deposition [19].

Karamifar et al. have demonstrated that 62.9% of girls and 69% of boys affected with thalassaemia were less than 2SD below the mean for normal height [20]. Sharma et al. studied an Indian cohort of beta-thalassaemic children on oral desferiprone, of whom 55% were of short stature and 27% had a height z-score less than −3 SD. In the subset with height z-score < −3 SD, 17 of 19 patients also had severely impaired GH induction in response to dynamic testing with clonidine [21]. In one cohort from Germany, 40.6% of patients were defined as being short in stature (final adult height < 3rd percentile/below 2 standard deviations [SD] from the mean) [22]. Soliman and colleagues replicated this observation, reporting short stature (<2SD) in 49% of their thalassaemic patients [23], whereas Borgna-Pignatti et al. reported short stature in 37% of their patients [24]. Moayeri et al. showed that

62% were less than 2SD and 49% were 3SD below the mean and also confirmed decreased growth hormone response to two provocative tests and low levels of IGF-1 in a majority of their thalassaemic patients[24]. Similar reduced responses to provocative tests have been reported in studies led by Gulati et al. (51%) and Theodiris et al. (20%) [10, 25]. Interestingly, Soliman et al. report that in a small sample of adult patients on oral chelation therapy, IGF-1 expression levels did not differ significantly between those with normal GH levels and those with GH deficiency, despite significantly lower final adult height in the GH deficient group [19].

Although the results of short term GH therapy are encouraging, the impact of treatment on final height of non-GH deficient thalassaemic children remains uncertain [18] and often GH produces uncertain clinical response [26, 27]. Ngim et al. have undertaken a Cochrane Systematic Review of GH replacement in thalassaemia. A single non-randomised trial was eligible, which enrolled 20 Turkish children with beta-thalassaemia major, receiving either daily subcutaneous GH or standard care. It presented tentative evidence that height velocity may be increased with GH, but reported no significant differences in the height standard deviations between groups at the study end-point [28]. Most patients lack the pubertal spurt and have reduced GH peak amplitude [29], hence responses to recombinant human GH therapy is poor when compared with that of children with GH deficiency, idiopathic short stature or Turner Syndrome.

The 2016 United Kingdom Thalassemia Society (UKTS) guidelines recommend stringent assessment of growth during childhood. This includes recording of height (both sitting and standing), weight and height velocity at six-monthly intervals until final adult height is attained [30]. Height deficits should prompt referral to a paediatric endocrinologist. Plain hand/wrist radiographs at 1–2 year intervals until fusion of the epiphyses may aid investigation of faltering height velocity [30]. Reduced height velocity, particularly around age 8–12 years, should prompt consideration of both desferrioxamine toxicity and GH deficiency, requiring GH stimulation testing and supplementation if deficient [30].

In contrast to GH deficiency in childhood, GH abnormalities in adults with thalassaemia are less well characterised. Recent data from an I-CETA survey (International Network of Clinicians for Endocrinopathies in Thalassemia and Adolescent Medicine) covering 3314 adult thalassaemia major patients across 15 international centres, reported a GH deficiency incidence of 3% [31]. The discrepancy between this figure and earlier estimates from paediatric cohorts is multifactorial. Unlike childhood growth failure, there is no obvious pathological correlate of adult growth hormone deficiency to prompt investigation. Adult GH deficiency can manifest with neuropsychiatric symptoms; abnormal body composition; and cardiac features, including both reduced exercise performance [32] and altered myocardial structure [33]. Soliman and colleagues have proposed criteria for GH deficiency screening in adults with thalassaemia major. These include individuals with high iron loads, short stature (height < −2.5 SDS), low serum IGF-1 (< −2 SDS) or existing cardiomyopathy [34]. Given increasing survival of patients with thalassaemia major into adulthood, this topic remains in need of further investigation.

3. Hypogonadism and puberty in thalassaemia

Sexual immaturity is a profound complication of severe thalassaemia [35]. Disruption of the hypothalamic–pituitary-gonadal axis (HPG) may result in infertility [36]. While hypogonadism can occur as a result of primary or secondary hypogonadism or as a combination of both, multiple studies have shown

gonadotropin failure (hypogonadotropic hypogonadism) is the commonest complication [36, 37]. Primary gonadal failure is caused by iron deposition on gonadal tissue [37]. Secondary hypogonadism occurs as a result of pituitary gland gonadotrophic cell iron deposition, as evidenced by GnRH stimulation which demonstrates inadequate FSH and LH response [38–40]. Failure of pubertal onset has a very high incidence rate, with studies varying between 50–100% [9]. In female patients, delayed puberty is defined as a lack of breast development in girls by age 13 and in male patients, by a lack of testicular development by age 14 [30].

Delayed puberty in patients with beta thalassaemia major occurs as a result of multiple factors. Evidence suggests that the accumulation of excess iron from multiple transfusions leads to tissue damage in multiple organs (e.g. the liver, heart, endocrine organs), and the presence of free radicals leads to oxidative stress [41]. Delays in sexual maturation has been shown to be a result of impaired synthesis of leptin caused by the deposition of iron on adipose tissue [42]. Adipose cells response to the expression of the *ob* gene to produce leptin which functions as an indicator to instigate puberty. Despite chelation therapy, iron accumulation continues to occur in the pituitary, hypothalamus and gonads [43]. The lack of response to gonadotropin releasing hormone in patients with low gonadotropin levels is synonymous with hypothalamic and pituitary damage [44].

While MRI assessment of the pituitary gland for iron accumulation has been studied with promising results, it is currently not part of routine assessment [45]. In terms of growth velocity, patients with thalassaemia were found to have distinctly lower or completely absent annual growth rates [46]. Short stature was found in up to 20% of such patients [14] and the lack of pubertal growth spurt in puberty, whether spontaneous or induced, ultimately adversely affected the attainment of a normal final height [18]. Impairment of truncal growth [47] is also compounded by disproportionate body ratios and variation in spinal growth. As a result, failure of pubertal growth spurt, delayed or absence of sexual development and infertility are common sequelae among patients with beta thalassaemia major [36].

Hypogonadism as an endocrine complication in patients with thalassaemia major has been reported in multiple studies [36]. A high incidence of hypogonadotropic hypogonadism has been found by Chern et al. in their study population [48]. A 45% prevalence was found in male patients and 39% in female patients, with an overall prevalence of 72%. Of note were significant delays or cessation in development of secondary sexual characteristics and the menstrual cycle. These findings were reiterated in a cross-sectional study set up in Hong Kong, in which 75% of female patients and 62% of male patients were found to have diminished gonadal function [13]. In a study conducted of by Saffari et al., hypogonadism was found to be the most common endocrine complication. From a study population of 77 patients, 36 (46.8%) patients were found to have hypogonadism, 28 (36.4%) had delayed puberty and in 8 (10.4%) patients there was absence of pubertal progression [49]. In this study, it was also noted that there was 'significant correlation between bone mineral density and pubertal status ($p = 0.001$). This study demonstrates the effects of hypogonadism not only on the reproductive system, but also on bone mineral density as well [49].

Failure of puberty was reported by Moayeri et al. in 69% of patients with thalassaemia with suppressed FSH and LH levels (73.2% in male patients and 64.8% in female patients) [50]. Similar findings were reported in a separate Italian multicentre study, which described hypogonadism 47% of female patients and 51% of male patients. Hagag et al. demonstrated that in males testosterone levels and testicular volume were significantly lower in thalassaemic patients with iron overload [51]. A similar study conducted by Hagag et al. in female patients, they showed that FSH, LH and oestrogen levels were significantly lower in thalassaemic patients with iron overload [52].

Pubertal failure has also been described in 73% of male and 42% of female patients under the age of 21 years old by Soliman et al. [23]. Similar findings were noted by Borgna-Pignatti and colleagues with 67% of males and 38% of females experiencing puberty failure [24]. Remarkably, successful conception may still be achieved by women who receive adequate chelation therapy.

Bone growth, growth velocity and puberty may be assisted by administration of chelation therapy before the commencement of sexual maturation and the use of low dose sex steroids in adolescence [9].

Current Standards for the Clinical Care of Children and Adults with Thalassaemia UK Guidelines recommend that assessment of gonadal function should be done annually throughout adulthood [30]. In men, this would include an annual morning testosterone level alongside LH/FSH and SHBG if testosterone levels are low. In women, no testing is required in the presence of a normal menstrual history. However, in the presence of menstrual disturbances, further testing (LH/FSH and oestradiol) is recommended [30]. Deficiency should be replaced with oestrogen/testosterone while bearing in mind that under-replacement contributes to a low bone mineral density. Management is best achieved through a joint multidisciplinary consultation between the endocrinologist and thalassaemia team [30].

4. Glucose intolerance and diabetes mellitus

Effective management of patients suffering from homozygous beta thalassaemia has led to improved life expectancy and hence manifestations of haemosiderosis related complications, notably, disturbances of the exocrine and endocrine function of the pancreas [53]. The prevalence of glucose intolerance and diabetes mellitus (DM) in patients with homozygous beta thalassaemia has been found to be variable. A retrospective analysis of 92 patients performed by Ang et al. showed that diabetes mellitus was one of the most common endocrinopathies with 41% of the study population affected [54] while a study conducted by Kanbour et al. reported a prevalence of 16.7% for impaired fasting glucose and 12.5% for diabetes mellitus [55]. Recently, a meta-analysis conducted by He et al. which included 44 studies with 16,605 cases showed that diabetes mellitus was present in 6.54%, impaired fasting glucose (IGF) in 17.21% and impaired glucose tolerance (IGT) in 12.46% [56]. While the prevalence of glucose intolerance and diabetes mellitus is undoubtedly high, many risk factors have also been identified. There was evidence of increased risk of diabetes mellitus with co-infection with hepatitis C [55–57], longer duration of disease [57–59] and with increased pancreatic iron deposition [58, 60].

Li et al. have found that in addition, patients with diabetes mellitus were characterised by higher ferritin levels, smaller pancreas volume, lower cardiac T2 magnetic resonance signal (MRI) than patients without diabetes, and higher prevalence of hypogonadism. Interestingly, patients with diabetes were found to be young (median age was 22 years [range of 10 to 34 years]) and non-obese (BMI of 20.1 ± 2.8 kg/m^2) [58]. This may explain why the classical association between diabetes and increased prevalence of arteriosclerotic cardiovascular disease is not a feature in this population [30].

Poor compliance with desferrioxamine therapy ($p < 0.05$), older age commencing intensive chelation therapy, liver cirrhosis and severe fibrosis were found to be risk factors for glucose intolerance and diabetes mellitus. Risk factors for impaired glucose tolerance (IGT) also included male sex [61], poor compliance with desferrioxamine therapy and high hepatic iron concentration.

Current UK guidelines recommend annual monitoring for impaired glucose tolerance and diabetes from the onset of puberty, or from the age of 10 years if

there is a family history of diabetes [30]. Screening is carried out with the oral glucose tolerance test (OGTT) [30]. However, OGTT compliance is often poor. This makes the development of adjunct or alternative screening tests of particular interest, as detecting pre-clinical diabetes is crucial because the development of clinical diabetes can possibly be slowed down or halted. Pancreatic iron overload can be assessed by MRI [62] but does not seem to correlate with siderosis in other organs. Currently, the relationship between MRI detectable iron and pancreatic beta cell dysfunction is not well characterised and MRI of the pancreas for iron deposition monitoring is not used clinically [30, 63]. However, there may be scope to use cardiac and liver MRI which already have established protocols, for the purpose of screening for impaired glucose tolerance or diabetes. Ang et al. found that abnormal myocardial T2 signal may indicate the development of diabetes mellitus and other prediabetic states [54]. Li et al. showed similar findings whereby Cardiac T2 MRI values were higher in patients with normal fasting glucose levels ($P = 0.03$) [58]. Kanbour et al. found that patients with very high liver iron concentration (LIC) (>30 mg Fe/gm dry liver) were more likely to have a higher prevalence of impaired fasting glucose when compared to those with lower LIC ($p = 0.044$) [55]. The use of continuous glucose monitoring (CGMS) in detecting glucose intolerance and diabetes mellitus has also been studied, with CGMS found to be superior when compared to OGTT ($p = 0.012$) [64]. El-Samahy et al. studied 20 beta thalassaemia patients with random blood glucose >7.8 mmol, who then had OGTT and CGMS. OGTT detected 6/20 patients (30%) who had impaired glucose tolerance and 7/20 (35%) patients who were in the diabetic range, while CGMS found that 7/20 (35%) patients had IGT and 13/20 (65%) had frank diabetes mellitus [64].

In terms of determining overall glycaemic control, UK guidelines recommend that serum fructosamine should be used. Fructosamine is a circulating glycated protein which measures overall glucose control in the previous 2–3 weeks. HbA1c or glycated haemoglobin should be avoided in thalassaemia as it is unreliable in any haemoglobinopathy and also after transfusion [30].

Although inadequate insulin release has been reported by several groups [60, 65, 66]. Other aetiologies identified include hyperinsulinemia, reduced insulin sensitivity [67] and reduction of hepatic insulin release. A study by Siklar et al. suggested that development of insulin impairment occurs prior to insulin resistance [68]. Furthermore, autoimmunity results in selective oxidative damage to beta cells of the pancreas [68]. Beta cells retain their function up to the later stages of the disease [9], however insulin sensitivity was found to be inversely related to iron overload and age [69]. Fasting pro-insulin and pro-insulin to insulin ratios was found to be considerably elevated and have a positive correlation with hepatic iron [70], however C-peptide levels were found to be inconsistent, thus reflecting fluctuating beta cell function [71, 72]. A reduction in serum trypsin and lipase levels were found, alongside regular alpha amylase activity. It was also found that the development of other endocrine and cardiac complications were followed by the onset of diabetes mellitus [73]. A 50% decline beta cell function was found to be correlated with glucose intolerance, and beta cell function was not entirely recovered even after intensive iron chelation. Moreover, high transfusion regimes that were not paired with appropriate iron chelation could advance the prevalence of diabetes mellitus further.

The prevalence of abnormal glucose metabolism has gradually increased over the last 20 years [55]. Therefore, the topic of glucose intolerance and diabetes mellitus in patients with thalassaemia major continues to be of significant clinical importance.

5. Thyroid dysfunction

Thyroid dysfunction is a frequently occurring endocrine complication in thalassaemia major [39]. Hypothyroidism occurs either as a results of primary gland failure, or insufficient thyroid gland stimulation [74]. Hypothyroidism is thought to be a graded phenomenon and many types of hypothyroidism have been described: (1) sub-biochemical hypothyroidism: which consists of an exaggerated TSH response to TRH test in the presence of normal TSH and FT4; (2) sub-clinical hypothyroidism: elevated serum TSH with normal serum FT4 levels; (3) overt (clinical) hypothyroidism: High TSH with low FT4 level and (4) central Hypothyrodism: an inappropriately low or normal TSH with a low free T4 level [74]. The lack of autoimmune thyroiditis in thalassaemia patients continues to be supported by multiple studies [75, 76].

Subclinical hypothyroidisim was found to be the most prevalent thyroid dysfunction in many studies [77, 78]. In a study of 144 thalassaemia patients by by Saleem et al., hypothyroidism was found in 31.2% of patients. Subclinical hypothyroidism was found to be the most prevalent thyroid dysfunction (31.2%; 45 patients), whilst only 6.7% [3] patients were found to have overt hypothyroidism. Interestingly, 76% of the patients with subclinical hypothyroidism were in the first decade of life [77].

The study conducted by Yassouf et al. demonstrated that out of the 82 cases of thalassaemia studied, subclinical hypothyroidism was once again found to be the most prevalent thyroid disorder - 29.27% of patients [24] had subclinical hypothyroidism while only one patient (1.22%) had overt hypothyroidism. In contrast to other studies, no case of central hypothyroidism was found [78].

There is a general consensus that central hypothyroidism is underestimated as there are only a handful of studies on the topic currently. The diagnosis of central hypothyroidism remains difficult from a clinical perspective, as its non-specific symptoms means that symptoms are usually attributed to another cause. From a biochemical perspective, central hypothyroidism is diagnosed based on a low to normal TSH level, in the presence of low levels of free T4 [74].

De Sanctis et al. explored the prevalence of central hypothyroidism in their thalassaemia population (339 patients). They found that central hypothyroidism was present in 6% of patients aged less than 21 years old, and 7.9% in patients above 21 years of age. Delaporta et al. showed that 16% of 114 studied patients (mean age 20.9 ± 7.8 years) had central hypothyroidism [79].

A prospective study carried out by Soliman et al. followed a total of 48 patients over a period of 12 years. In this duration, hypothyroidism was diagnosed in 35% [17] of patients - central hypothyrodisim was found in 13/17 (76%) patients [75]. Unexpectedly, this paper also found that the mean serum ferritin level did not differ significantly between patients with or without central hypothyroidism. This in turn did not support the hypothesis that iron overload of the HPA axis had resulted in central hypothyroidism thus concluding that the precise underlying aetiology of central hypothyroidism was unclear. However, due to the susceptibility of the pituitary gland to excess iron, central hypothyroidism due to iron overload of the HPA axis still remains a possibility [74].

Belhoul et al. found an increase in prevalence of hypothyroidism in splenectomised patients [80]. In non-splenectomised thalassemic patients, the spleen was thought to have a scavenging effect on free iron fractions. However, further studies are needed to evaluate this hypothesis [74].

Thyroid failure was found to correlate with age at which iron chelation therapy started. When iron chelation therapy was started late, thyroid dysfunction was

found to occur earlier [74]. A study published in 2018 by Upadya et al. showed that of a population of 83 children with thalassaemia, 4.8% had evidence of subclinical hypothyroidism. In this study, the mean ferritin level was 3983.0 ± 169,830 ng/ml. However, while the severity of thyroid dysfunction was statistically significantly associated with higher serum TSH value in children in the second decade of life ($p = 0.001$), it is important to note that no significant correlation was found between the severity of thyroid dysfunction and serum ferritin levels [76].

These findings were also echoed in the study conducted by Yassouf et al. They found that serum ferritin was directly correlated with TSH levels ($r = 0.414$; $p < 0.001$). However, there was no correlation between serum ferritin and FT4 levels ($r = 0.027$; $p > 0.05$) [78]. This study also demonstrated that the risk of thyroid dysfunction was increased by non-compliance of chelation therapy by 6.38 fold as compared with compliant patients (RR = 6.385 l 95% CI, 2.40–16.95) [78].

In another study a total of 72 thalassaemia patients were followed for 8 years. The study endpoint was defined as the incidence of thyroid dysfunction, and aim of the study was to analyse ferritin as a prognostic maker. It found that that patients with thyroid dysfunction had higher ferritin levels in contrast to those with normal thyroid function (1500 (872–2336)) vs. (513 (370–698) ug/l; $P < 0.0001$). The study also found patients with ferritin values above 1800ug/L had a more rapid progression towards the endpoint of thyroid dysfunction [81].

However, as a single value, ferritin may not always be reliable. Ferritin, as an acute phase protein, is subject to fluctuations caused by other variables such as inflammation and malignancy. However, ferritin may still be the most convenient way to assess iron overload, especially when used as part of a serial measurement [81]. Ferritin may be of value as a prognostic maker and may be used to identify patients at risk of developing thyroid dysfunction [81]. This begs the question as to whether the value of ferritin in determining the severity of thyroid dysfunction is over-appreciated [76].

Currently, the UK Thalassaemia Society Standards for the clinical care of children and adults with thalassaemia in the UK (2016) recommend thyroid functions tests annually in patients with thalassaemia from age of 12 years, or if there are any suggestive symptoms of thyroid deficiency between times [30].

6. Hypoparathyroidism

Hypoparathyroidism, resulting in hypocalcaemia, is a late complication of iron overload, typically manifesting after the age of 10 years and with a higher incidence in men [46]. The first sign of incipient hypoparathyroidism is loss of the diurnal pattern of parathyroid hormone (PTH) secretion [9]. The typical biochemical profile is low serum calcium, low serum PTH, low serum vitamin D and elevated serum phosphate levels [82]. Clinical signs of the disorder are most frequently noted from the second decade of life onwards [15].

A recent survey of clinicians by the International Network of Clinicians for Endocrinopathies in Thalassemia and Adolescence Medicine (ICET-A) published in 2018 reported a prevalence of 6.8% among 3023 thalassaemia major patients from 17 centres [83]. 42.2% of hypoparathyroid patients in this study were described as asymptomatic at diagnosis. The most common presenting symptom was paraesthesia and/or cramping seen in 37.6% patients [83]. In the ICET-A study, 49.8% of [83] hypoparathryoid individuals were also noted to have serum ferritin level > 2.500 ng/ml[8]. Chirico and colleagues also noted a significant association between elevated ferritin and incidence of hypoparathyroidism in thalassaemia major, proposing its use a prognostic marker for development of endocrinopathy [84].

Inconsistencies in case definition of hypoparathyroidism have complicated estimates of prevalence. In a cohort of transfusion-dependent thalassaemia major patients, 13.5% were shown to have hypoparathyroidism, characterised by low serum PTH, total and ionised calcium levels [85]. In contrast, a multicentre study in Italy, encompassing 25 units, showed the prevalence to be 3.6% [46]. A French study from 1993 showed the prevalence of hyperparathyroidism to be as high as 22.5% [86]; similarly Aleem and colleagues reported a prevalence of 20% [87]. Shamshirsaz and colleagues showed a prevalence of 7.6% [1] with male:female ratio of 4:1, a higher ratio than has been described elsewhere [46, 83]. Further studies in Iran by Bordbar et al. and Bazi et al. reported prevalences of 13.2% [88] and 18% [89] respectively among their thalassaemia patient cohorts. Interestingly, Tangngam and colleagues reported a prevalence of asymptomatic hypoparathyroidism of 38% in their cohort of 66 transfusion-dependent thalassaemia patients, with significantly lower serum FGF-23, a major regulator of phosphate, detected in hypoparathyroid patients [90].

A small study by Even and colleagues suggests loss of normal diurnal variation in PTH secretion in thalassaemia patients, even in individuals with normal daytime calcium levels [91].

In the UK, annual screening of parathyroid function and bone profile is recommended in thalassaemia patients [30]. Limited data [92, 93] shows that early supplementation with Vitamin D or calcitriol treatment for three months is sufficient to normalise plasma calcium and phosphate levels. 2016 UKTS guidelines recommend treatment with activated vitamin D preparations in the case of primary hypoparathyroidism secondary to iron overload. In order to avoid nephrocalcinosis, adjusted calcium levels should be targeted towards the lower reference range [30].

With regard to other complications, tetany, seizures or cardiac failure due to severe hypocalcaemia is rare and requires immediate correction with intravenous administration of calcium. Koutsis and colleagues report a rare case of Fahr's syndrome—striatopallidodentate calcinosis—in a 42 year-old woman with thalassaemia major with erratic compliance with oral vitamin D/calcium supplementation. Her hyperkinetic symptoms resolved with resumption of adequate oral supplementation [94].

7. Adrenal dysfunction

Histological and imaging studies have shown that iron deposits in the adrenal cortex of thalassaemic patients are mainly confined to the zona glomerulosa with rare involvement of the zona fascicularis [95]. Most studies have revealed intact pituitary adrenal axis in thalassaemia patients [35, 60, 65, 66, 96]. Prevalence of adrenal insufficiency is variable and depends both on the degree of iron overload, cut off values for cortisol measurement and diagnostic test used [97, 98].

Poggi et al. used a low dose synacthen test with adrenal insufficiency determined by cortisol <500 nmol/L and found a prevalence of 13.7% in the study population [97]. Huang et al. used a glucagon stimulation test, followed by corticotrophin-releasing hormone and found a prevalence of 61% [98].

Raised ACTH levels were found by McIntosh which suggests primary adrenal failure [66], however Costin et al. found suppressed ACTH levels and reduced adrenal reserve despite the lack of clinical signs [5]. The diminished ability of the adrenal cortex to react to further pulses of ACTH may be reflected in the fact that baseline serum and urinary cortisol levels are usually normal [99].

Low serum Dihydroepiandrostenedione (DHEA), Dihydroepiandrostenedione Sulphate (DHEAS), androstenedione and testosterone levels were found to be

caused by the dissociation between androgen, cortisol and aldosterone synthesis. This may be the reason adrenarche is usually absent in these patients [100]. Dysfunctions in ACTH secretion circadian patterns but unaffected cortisol and aldosterone secretory were seen in these patients [101]. In addition to that, falsely low serum cortisol levels may be found in thalassaemic patients with chronic liver disease because cortisol is usually bound to cortisol binding globulin (CBG) which is produced by hepatocytes [102]. Currently, there are no reports on CBG levels in thalassaemic patients. Nonetheless, the role of CBG in adrenal insufficiency is excluded by a normal CBG level in the presence of low cortisol. In female patients, oestrogen may cause a rise in CBG levels resulting in inaccurate cortisol levels.

Current research shows female gender to be a protective factor [97]. Huang et al. have found that there was a significant prevalence of adrenal insufficiency in males when compared to females (92% vs. 29%, $p = 0.049$) in their study cohort [98]. Imaging studies by Drakonaki et al. using MRI scan have frequently identified adrenal hypointensity without alteration of morphology in thalassaemia patients and verified autopsy findings of correlation between adrenal iron and liver iron [103]. However a study by Guzelbey et al., found that, on the contrary, there was no statistically significant correlation between iron deposition in the adrenal glands and liver [104]. Another study suggests that imaging from Cardiac MRI T2 could be used as a surrogate of adrenal hypofunction, with a sensitivity of 81% and specificity of 78% [105]. However, despite high sensitivity, histology still remains the gold standard for diagnosis of iron deposition.

Currently, routine cortisol monitoring does not form part of the recommended routine investigations to screen for adrenal dysfunction in patients with thalassaemia [30]. However, the UK standards for thalassaemia guidelines acknowledges that annual monitoring of cortisol may allow for trends in decline to be noted, but also stress that normal cortisol levels does not exclude partial adrenal insufficiency when the patient is unwell. While current literature is very contradictory, adrenal dysfunction can be life-threatening in an acutely unwell patient. Therefore, hydrocortisone supplementation should be considered even before formal proof of insufficiency is available, if clinically relevant [30].

8. Osteoporosis

Beta thalassaemia is associated with marrow expansion, osteopaenia with cortical thickening, trabecular coarsening and bone deformity [106]. Osteoporosis—defined as a microarchietctual deterioration in bone tissue leading to an increased fracture risk [107]—is the predominant bone disease in beta thalassaemia. The prevalence of osteoporosis in thalassaemia is variably estimated from 13.6–50% [108]. In a cohort of well-treated thalassaemia patients, Baldini and colleagues reported demineralization—.osteoporotic or osteopaenic bone—in 92.7% [109].

Factors implicated in its cause include hypogonadism, diabetes mellitus, hypothyroidism, hypoparathyroidism, iron overload and its treatment [108, 110]. Malnutrition, inadequate exercise and absence of adrenal sex hormones during adrenarche and gonadal hormones during puberty are other contributory factors [111]. Excessive erythropoesis may also impair bone formation [112]. Iron chelation therapy is further linked to hypercalciuria, with consequential nephrolithiasis and reduced bone mineral density (BMD) [113]. Desferrioxamine is also linked to bone dysplasia, independently of osteoporosis [114].

Spine and hip osteoporosis is common in both sexes, with spinal osteoporosis more common in women and the lumbar vertebrae and femoral neck affected more frequently in men [110]. Osteoporosis is characterised by significant decreases

in bone mineral density, both cortical and trabecular. In an Italian case series, pathological fractures were reported in 19.7% of transfusion-dependent patients with thalassaemia major [115]. With regard to putative mechanisms, significantly lower osteoprotegerin/RANKL ratios have been observed in thalassaemia patients. Excessive RANKL activity favours osteoclastic bone resorption, leading to reduced BMD [116]. Sapunarova and colleagues report 10-fold higher serum levels of sclerostin, a secreted glycoprotein with anti-osteoblastic properties, in adults with transfusion-dependent beta thalassaemia compared to controls [117]. Given its correlation with both lumbar spine/femoral neck Z-scores and incidence of fragility fractures, it has been proposed to act as a biomarker of severe osteoporosis in advanced beta thalassaemia. In Tehran, Shamishiraz et al. [1] demonstrated similar prevalence of that osteoporosis and osteopaenia in the lumbar spine (50.7% and 39.4% respectively) of patients with thalassaemia. In the same cohort, osteoporosis and osteopaenia prevalences were 10.8% and 36.9% at the femoral neck. These prevalences have been replicated in other cohorts [118, 119]. Jensen amd colleagued reported "severely low" bone mass in 51% of thalassaemia patients, with "low bone mass" in 45% [110]. Among 31 thalassaemic patients studied by Vogiatzi and colleagues (5 of whom with the milder thalassaemia intermedia phenotype), 22.6% had reduced bone mass (defined as Z score = −1 to −2 on DXA analysis) and 61.3% had a low bone mass (Z score ≤ −2) [93].

Early diagnosis requires accurate estimation of BMD via densitometry. Interpretation of dual-energy X-ray absorptiometry (DXA) may be confounded in thalassaemia due to short stature and spinal deformities caused by medullary expansion, bone dysplasia and the increased rate of degenerative vertebral disc disease in TM patients [120, 121]. Artefact from hepatic iron loading might also derange DXA analysis [122]. Alternative modalities for assessing bone microarchitecture in thalassaemia include trabecular bone structure (TBS) analysis—a textural assessment derived from DXA images—and quantitative computed tomography (QCT). These alternative methods remain limited by the distinctive profile of thalassaemia osteopathy and, in the case of QCT, by the amount of vertebral iron deposition [121, 123].

Prevention, early diagnosis and effective chelation therapy are most effective in arresting the progression of bone disease in thalassaemia. Tight adherence to recommended chelation treatment is required during childhood to prevent desferroximine-asssociated bone pathologies, including "pseudo-rickets" and cartilaginous dysplasia [30]. Diets rich in calcium and Vitamin D and exercise can improve the outcome [69]. The role of lifestyle interventions is particularly prominent during childhood. If deficient, calcium, vitamin D and zinc supplementation are advised, preferably via the oral route [119]. 2016 UKTS guidelines advise that many patients will require maintenance vitamin D3 supplementation [30]. Annual monitoring of vitamin D levels are recommended from age 2 years, aiming for a level of approximately 80 nmol/L. Intramuscular depot injection of vitamin D are not recommended by UKTS, nor are activated vitamin D preparations (for example alfacalcidol) in the absence of proven hypoparathyroidism secondary to iron deposition [30].

In terms of anti-osteoclastic treatments, the human anti-RANKL monoclonal antibody denosumab has shown promise in a Phase 2b RCT, increasing both lumbar spine and wrist BMD in transfusion-dependent TM patients [119]. Data from both Indian and Iranian cohorts suggest a role for zolendronic acid in increasing lumbar spine BMD [124, 125]. Alendronate and vitamin D regimen showed promise in an Italian Phase 2b RCT [126]. Prior to this, Morabito and colleagues showed that daily oral alendronate increased BMD in a two-year study of young adults with thalassaemia [127]. A 2016 Cochrane review of both bisphosphonates and zinc

supplementation for thalassaemia-associated osteoporosis acknowledges an accretion of evidence in favour of their use, but suggests that further RCT evidence is required [128].

Current UKTS guidelines advocate consideration of anti-osteoclastic agents for individuals with low age-adjusted BMD or in whom fragility fractures have occurred despite appropriate vitamin D/calcium or hormone supplementation. Bisphosphonate initiation should occur following consultation with a specialist in osteoporosis, given that no definite evidence exists for fracture reductions with bisphosphonates in thalassaemia patients, despite the evidence for improved BMD. Burden of adverse effects, including atypical femoral fractures and osteonecrosis of the jaw, are significant [30].

Hormone replacement therapy is beneficial in patients with concomitant osteoporosis and hypogonadism but may not offer complete resolution, due to the multifactorial nature of bone pathology in thalassaemia [129, 130]. Patients with concomitant hypogonadism require hormone replacement therapy [108].

9. Conclusions

There is a high incidence of endocrinological abnormalities in patients with thalassaemia. Thalassaemic patients have been shown to have an elevated frequency of endocrinopathies in several research centres. The role of regular follow-up to allow early detection and proper management of complications is vital. The care and quality of life of thalassaemic patients can be positively impacted by advancements in transfusion protocols and chelating therapy. Early recognition of endocrinopathies in patients with thalassaemia is crucial apart from the fact that life expectancy is increased, at the same time morbidity and mortality as a result of complications can be decreased with routine monitoring, appropriate interventions and follow-up in thalassaemia-endocrine join care clinics.

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